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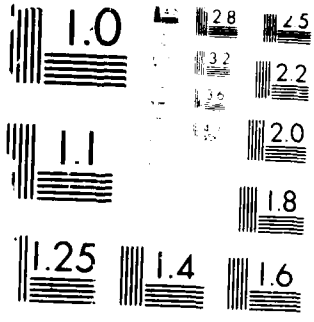
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Author(s) Candace B. Matthew, Roger W. Hubbard, Ralph P. Francesconi, and Glenn J. Thomas

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We have reported that when rats (500 g, male) are exercised to exhaustion on a treadmill, pretreatment with the centrally acting carbamate physostigmine reduced endurance (run time, RT) and increased the rate of rise of core temperature (Tc+). Both RT and Tc+ were restored to control levels by pretreatment with either or a combination of atropine (A), and diazepam (D). Our objective in the present work was to determine whether A+D could also restore the performance and thermoregulatory decrements induced by the peripherally acting carbamate pyridostigmine (PY). After drug administration, rats were run (11m/min, 6° elevation, Ta= 26°C) to exhaustion. PY treatment resulted in a reduced RT and an increased heat gain that neither A nor D alone (A+PY and D+PY) could restore to control levels. On the other hand, a combination of both A and D restored these variables to control levels. In conclusion, A+D can restore the performance and thermoregulatory decrements resulting from the administration of either a centrally or a peripherally acting carbamate.

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CARBAMATES, ATROPINE, AND DIAZEPAM: EFFECTS ON PERFORMANCE IN THE RUNNING RAT

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SUMMARY

We have reported that when rats (500 g, male) are exercised to exhaustion on a treadmill, pretreatment with the centrally acting carbamate physostigmine reduced endurance (run time, RT) and increased the rate of rise of core temperature (Tc↑). Both RT and Tc↑ were restored to control levels by pretreatment with either or a combination of atropine (A), and diazepam (D). Our objective in the present work was to determine whether A↑D could also restore the performance and thermoregulatory decrements induced by the peripherally acting carbamte pyridostigmine (PY). After drug administration, rats were run (11m/min, 6° elevation, Ta= 26°C) to exhaustion. PY treatment resulted in a reduced RT and an increased heat gain that neither A nor D alone (A↑PY and D↑PY) could restore to control levels. On the other hand, a combination of both A and D restored these variables to control levels. In conclusion, A↑D can restore the performance and thermoregulatory decrements resulting from the administration of either a centrally or a peripherally acting carbamate.

The carbamates, a group of anticholinesterases, are used clinically for the treatment of myasthenia gravis (2) and anticholinergic syndrome (9). Additionally, pyridostigmine (PY), a quaternary carbamate, is being considered for fielding by the military as a prophylactic against nerve agent poisoning (4, 10, 13). Because of the potential of these drugs to elicit cholinergic overstimulation, we have been concerned with their physical, physiological, and thermoregulatory effects during exercise in a warm environment. Heat dissipation in man is primarily accomplished by the evaporation of sweat from the body surface; analogously, rats secrete and spread saliva over their ventral surface for evaporative cooling (11, 15). Both sweat and saliva secretion are cholinergically regulated (9); therefore, an anticholinergic such as atropine would be expected to decrease salivation and the anticholinesterases to increase salivation.

We have previously used the sedentary heat-stressed rat to determine relative anticholinergic and anticholinesterase drug potency (14). This model has been extended to include the exercising rat as a performance based measure of drug effects (16). In such experiments, rats that exercised to exhaustion on a treadmill after administration of the carbamate physostigmine salicylate (PH), displayed a reduced endurance capacity (run time, RT) and an increased rate of rise of core temperature (Tc↑). The decrements in both endurance and thermoregulation were restored to control levels by the administration of atropine (A) and diazepam (D).

Like A and D, physostigmine, a tertiary carbamate, is able to cross the blood brain-barrier and therefore has central effects. Other work from this laboratory has demonstrated that the acute administration of the peripherally acting carbamate PY, in a dose that inhibited plasma cholinesterase by 65%, also resulted in reduced performance and increased rate of heat gain during exercise in the heat (6). However, when the PY was administered chronically in a dose that inhibited plasma cholinesterase by less than 40%, there was no decrement in either RT or Tc+ (7). Therefore, the present work was undertaken to determine if there is a performance or thermoregulatory deficit after PY is administered acutely to rats exercising in a moderate environment to inhibit cholinesterase by 40% and, further, to determine if A+D are able to restore anticipated performance decrements to control levels.

METHODS

Eight groups of 10 adult male Sprague-Dawley rats (Charles River, CD strain, 510-530 g) were used only once. The animals were housed individually in wire-bottomed cages and maintained in an environmental chamber at 26°C and 50% rh. Lighting was controlled automatically (on, 0600-1800 h) and Purina rat chow and water were available ad lib except during experimental intervals.

Prior to running, each rat received 3 separate injections at 10 min intervals via a lateral tail vein. The drugs, doses, and order of administration for each of the 8 groups are presented in Table 1. Atropine (A, 200 ug/kg, as the sulfate, Sigma Chemical Co.) was dissolved in 0.2 ml sterile 0.9% saline; diazepam (D, 500 ug/kg, Valium^{R}, Hoffman-LaRoche Inc.) was diluted to 0.5 ml with fresh rat serum; and pyridostigmine bromide (PY, 400 ug/kg, Mestinon^{R}, Roche Laboratories) was diluted to 0.2 ml with saline. Each drug dose used is within the human clinical range for the respective drug when the formula of Freireich et al (8) is applied (comparable rat dose = 7x human dose on a per kg basis).

TABLE I

Drugs, Doses, and Order of Administration

DRUGS

C vehicle control - 0.2 ml saline + 0.5 ml serum +0.2 ml saline
A atropine - 200 ug/kg in 0.2 ml saline
D diazepam - 500 ug/kg in 0.5 ml serum
PY pyridostigmine bromide - 400 ug/kg in 0.2 ml saline

	INJECTIONS*		
GROUP	1st	2nd	3rd
C	SALINE	SERUM	SALINE
A	A	SERUM	SALINE
D	SALINE	D	SALINE
A+D	A	D	SALINE
PY	SALINE	SERUM	PY
A+PY	A	SERUM	PY
D+PY	SALINE	D	PY
A+D+PY	A	D	PY

* 10 min apart, via lateral tail vein

Fifteen min after the final (3rd) injection the rats were weighed and then fitted with thermisters to measure core temperature (Tc, 6.5 cm into the rectum) and tail skin temperature (Tt, midlength, dorsum); then they were placed on the treadmill (shock avoidance contingency). The rats were run (11 m/min, 6° incline) at an ambient of 26°C and 50% rh until they were exhausted (unable to right themselves when placed on their backs). At exhaustion the animals were removed from the treadmill and allowed to recover. During the run and recovery Tc and Tt were monitored and the shocker (maximum output = 8 ma) on the treadmill was controlled by a HP9825 computer-controlled data acquisition system (17).

The data were analyzed by a one-way analysis of variance followed by the Student-Newman-Keuls multiple range test for all pair comparisons. The null hypothesis was rejected at the 0.05 level.

RESULTS

Fig. 1 demonstrates the effect of the various pharmacological interventions on endurance and rate of increase of core temperature. The PY group (40% inhibition of plasma cholinesterase, data not shown) manifested a very high Tc• and a correspondingly short endurance time. In contrast D alone or in combination with A reduced the rate of rise of core temperature with corresponding improvement in run time. Atropine alone at the dose given did not alter either run time or heating rate. The positive ergogenic effect of diazepam alone noted above could not overcome the overwhelming effect of pyridostigmine on heating rate during exercise. Also, the effect of the

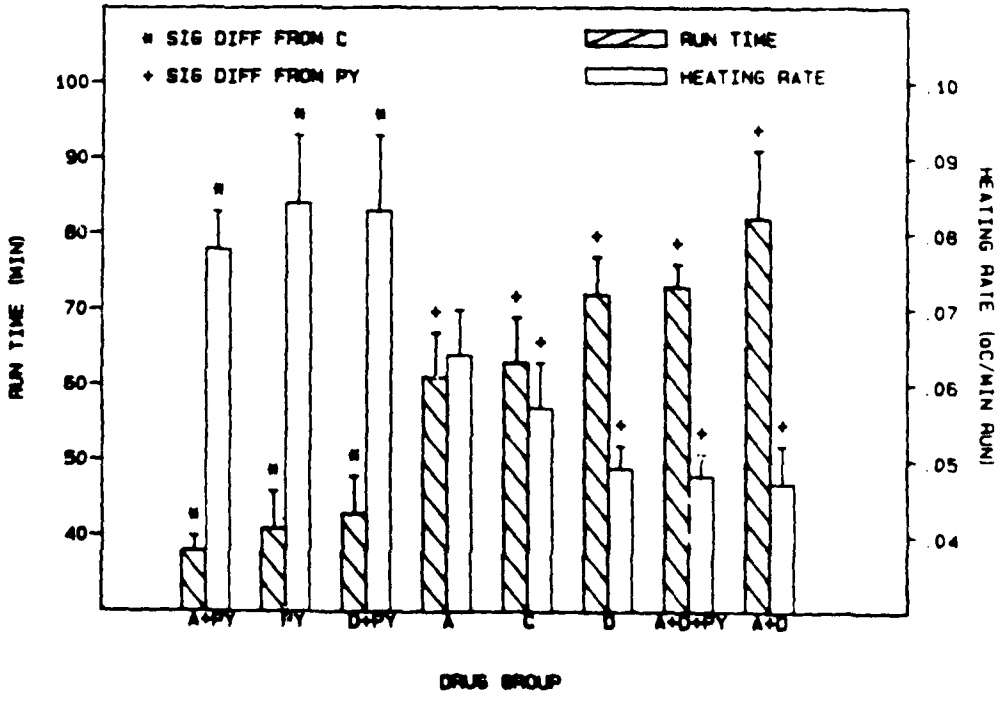

FIG. 1
Run time and heating rates (rate of rise of core temperature) for each of the drug groups (see Table 1 for identification of groups). Values are mean ± SE, * and + indicate a significant ($p<.05$) difference between the values and the C or PY groups, respectively.

peripherally acting carbamate PY on heating rate and performance could not be blocked by the centrally and peripherally acting anticholinergic A. But, the combination of A and D did restore both thermoregulatory and physical performance in the A+D+PY group to control levels.

TABLE II

Work Done and Weight Loss Rates for Running Rats

	DRUG GROUPS							
	C	A	D	A+D	PY	A+PY	D+PY	A+D+PY
WORK (kg.m)	37.5+ ±3.2	35.8+ ±3.5	42.7+ ±3.2	48.6+ ±5.5	23.8* ±2.6	22.5* ±1.3	25.0* ±2.8	42.6+ ±2.0
WT LOSS (g/min)	0.33+ ±0.02	0.21*+ ±0.02	0.29+ ±0.01	0.18*+ ±0.02	0.40* ±0.02	0.27+ ±0.02	0.31+ ±0.02	0.22*+ ±0.01

Values are mean + SE
* significantly different ($p<.05$) from C
+ significantly different ($p<.05$) from PY

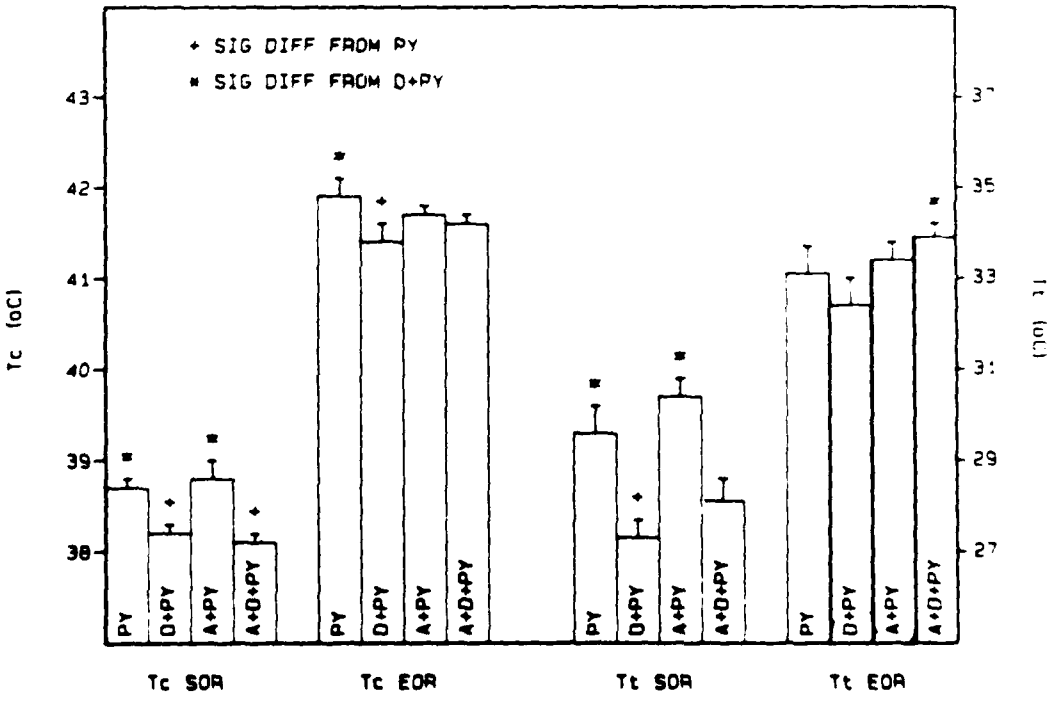

FIG. 2
Core (Tc) and tail (Tt) temperatures at the start of run (SOR) and end of run (EOR) for the 4 groups receiving pyridostigmine (see Table 1 for groups). Values are mean + SE, + and * indicate significant ($p<.05$) differences between the values and PY or D+PY groups respectively.

Run time and work done are not always comparable; therefore, a useful measure of the work done (Table 2) when comparing animals of different sizes is the kg.m (kg.m = body wt (kg) x run time (min) x speed (m/min) x treadmill inclination (sin)). As anticipated when all the animals are the same size and run at the same speed and inclination, the work done increased with RT (Fig. 1), and the PY, A+PY, and D+PY groups all did significantly less work than the other groups. Weight loss during the treadmill run is the total weight lost through urination, defecation, salivation, and respiration. Also in Table 2, wt loss data is presented in terms of wt loss per min of run. The wt loss rate for the A, A+D, and A+D+PY groups are all significantly lower than the control group as a result of the antimuscarinic effects of atropine on salivation and defecation Alternatively, the PY group had a wt loss rate significantly higher than any other group; since PY may stimulate salivation and defecation. Diazepam alone did not have any effect on wt loss rate, but A or D, when administered alone or in combination, significantly reduced the PY-stimulated wt loss rate.

Figure 2 depicts the core temperature (Tc) and tail temperature (Tt) at the start of run (SOR) and the end of run (EOR) for the 4 groups receiving PY. Except for the A+D+PY group all of these groups had high heating rates and low endurance times (Fig 1).

DISCUSSION

The apparent inverse relationship between heating rate (rate of increase of core temperature) and run time of rats running on a treadmill (Fig 1) has been noted in earlier work from this laboratory (12, 16). Rats dissipate a significant amount of body heat by increasing blood flow to the tail (19), and this ability is particularly important in a running rat which is physically unable to spread saliva behaviorally for evaporative cooling due to the exercise contingency. Therefore, a difference in Tc or Tc+ of running rats mustd be explained by either a change in heat loss through the tail or a change in metabolic heat production. In Fig 2, the lower Tc SOR and EOR of the D+PY group indicate a thermoregulatory advantage of this group over the PY group, but the lower Tt in the D+PY group (vs PY group) resulted in less heat loss. Preliminary data indicate that D does decrease O_2 consumption in the running rat. In the A+PY group the disadvantage of a higher Tc would have been only partially compensated for by the higher Tt and greater heat loss from the tail. The A+D+PY group manifested the lower Tc of the D+PY group and the higher Tt of the A+PY group.

In our earlier work with physostigmine (PH) (16), the administration of A+D to PH-treated rats restored endurance and thermoregulatory performance just as A+D restored decrements resulting from PY administration. The doses of PY (400 ug/kg) and PH (200 ug/kg) used in these 2 experiments resulted in similar (40%) inhibitions of plasma cholinesterase. Despite these consistencies, the mechanisms of interaction of A and D with PH and PY may be different. In the present work, neither A nor D, when administered singly to PY-treated rats, was effective in improving endurance and thermoregulatory ability as indicated by the high heating rates and low run times of the A+PY and D+PY groups in Figure 1. However, in our earlier work (16) both A and D were able to restore the PH induced decrements in endurance and thermoregulation to control levels.

There are several characteristics of PH and PY which partially explain the divergent results of the present (PY) and the earlier (PH) experiments. PH, a tertiary carbamate, is able to traverse the blood-brain barrier and therefore has central sites of action. PY, in contrast, is quaternary and, therefore, does not have such sites of action. Both A and D apparently do have sites of action in the CNS as well as the periphery (9).

Centrally acting anticholinesterases induce a peripheral vasodilation resulting in a high Tt and concomitant lower Tc in the rat (18); the higher tail temperature at the start of run was present in all groups receiving PH (16). With peripherally acting PY, tail temperatures in the present study were not elevated over control levels. While the higher tail temperature of the PH-treated rats did not attenuate heating rate during exercise (16), it does indicate that the PH- and PY-treated rats may exhibit different thermoregulatory effects. In addition, both PH and PY have direct, but different, effects on the nicotinic acetylcholine receptors at the neuromuscular junction; at this site, PH is an open channel blocker, and PY is a weak agonist (1). Since atropine is also an open channel blocker with a higher affinity for open channels (5), the channel-blocking effect of PH may act synergistically with A, while the weak agonist property of PY would oppose the actions of A. Finally, D depresses central cholinergic neurons by blocking acetylcholine release (3); since this is only a central effect, it could help explain the beneficial effects of D in the PH group but not in the PY group.

The dose of D (500 ug/kg) used in both the PH and PY experiments is insufficient to lower Tc SOR (16); however, preliminary data indicate that this dose of D lowers O_2 consumption in both sedentary and running rats. This reduced metabolic rate should improve thermoregulation in any environment where heat storage exceeds heat dissipation as is certainly the case for rats running on a treadmill at an ambient temperature of 26°C. To date the carbamate treated rats have been run at ambient temperatures of 26°C or higher (6, 7, 16); the effect of lower ambient temperatures on the physical performance of carbamate treated rats should be determined. While the PY-treated groups demonstrated significantly greater salivary water loss than other groups (Table 2), this saliva could not be spread for cooling. Since the heating rates (Fig. 1) of the PY, A+PY, and D+PY groups are all significantly greater than the heating rate of the control group, exercise without evaporative cooling may have revealed a thermogenic effect of pyridostigmine administration (12).

Administration of the anticholinesterase pyridostigmine to running rats resulted in reduced endurance and an increased rate of rise in core temperature. Both effects were neutralized by pretreating the animals with a combination of the anticholinergic atropine and the anticonvulsant diazepam. Since we have previously reported similar results for physostigmine, this is a performance-based model system which may be used to examine the mechanisms of carbamates that do or do not penetrate the blood brain barrier.

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation. In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide of Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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